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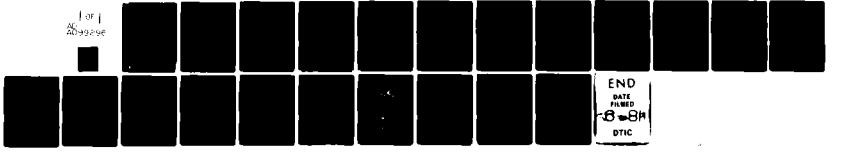

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MELBOURNE, VICTORIA

REPORT

MRL-R-781

MYTILUS EDULIS AS AN INDICATOR OF TRACE METAL POLLUTION IN NAVAL DOCKYARD WATERS WITH PRELIMINARY RESULTS FROM WILLIAMSTOWN NAVAL DOCKYARD, VICTORIA

Ian C. Dunstan, Albert de Forest and Russell W. Pettis*

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West Footscray, Victoria.

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C O N T E N T S

* * *

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1. INTRODUCTION

Contamination of the marine environment by trace metals can produce deleterious effects on upper levels of marine food chains, and subsequently man [1,2,3]. Assessment of trace metal loads in regions of effluent discharge ensures that contamination by trace metals can be detected before dangerous levels are reached.

The possibility of heavy metal input from dockyard activities has led authorities at Williamstown Naval Dockyard (WND) Victoria to request a study of trace metal levels in waters adjacent to the dockyard in Hobson's Bay.

Trace metal concentrations in the marine environment can be measured in water [e.g. 4,5], sediments [e.g. 4,6] or organisms [e.g. 7,8,9,10]. Measurements performed on marine organisms have the following advantages over the alternative sources :

(i) trace metal levels in organisms are higher and hence less subject to analytical error than those from sediments or water samples [5,7],

(ii) levels in organisms are buffered against short term fluctuations in ambient levels of trace metals and therefore reflect past environmental conditions more accurately than those gained from water samples [11], and

(iii) measurements from organisms provide data on the trace metal load in the marine food system not provided by measurements of water or sediment trace metal content [12].

Numerous marine organisms have been used to assess trace metal pollution. These include various algae [13,14], crustacea [12,15,16], ascidians [17,18], polychaetes [19], bivalves [20,21,22,23] and fish [24,25].

Barbaro [12] lists the following criteria required for an organism to be an effective pollution indicator :

(1) sessile living habits,

(2) availability in all seasons,

(3) ease of sampling,

(4) ubiquitous occurrence and abundance in the areas to be monitored,

(5) predisposition for a consistent uptake of one or more pollutants,

(6) high capacity for accumulation above environmental levels, and

(7) predisposition for retaining pollutants for a sufficient period after a reduction in environmental levels.

Mussels, particularly *Mytilus edulis*, have been used as pollution indicators in local [10,26,27] and overseas studies [28,29,30,31].

Mussels are sessile in habit and occur abundantly throughout the year in easily accessible locations at WND. They are also found in waters adjacent to Naval facilities in Sydney Harbout [32] and Cockburn Sound [33].

Experimental evidence [34] has shown the rate of Pb uptake by *Mytilus edulis* to be linearly dependent upon the concentration in the surrounding medium. Uptake of Cu, Pb, Co, Cd and Ni by *Mytilus edulis* has been quantified under experimental conditions [34]. The mussel has been considered to reflect ambient levels of Zn [10,26,29,34,36], Pb [1,10,26,29,34], Ni [35], Ag [29], Cu [1,29], Cd [1,10,26], Cr [29], Mn [10,36], Fe [10,36] and Co [36], although other writers express doubts on the ability of *Mytilus edulis* to monitor environmental levels of Ni and Cu which show atypical uptake behaviour in molluscs [26,29]. The Zn, Cd and Pb content of mussels have been shown to reflect industrial effluent discharge in Port Phillip Bay [26]. Retention of metals for varying periods of time after termination of supply has been demonstrated experimentally for Cd, Co, Ag [1] and Pb [1,34].

Mytilus edulis therefore satisfies the necessary criteria for indicator organisms and can be used to assess trace metal levels in dockyard environments. Trace metal contamination can be measured for the whole organism [10,26,30,31,37] or in specific organs [27,28,29]. Analysis of the various organs affords additional data on the uptake pathways of trace metals, although *in situ* information needs to be supplemented with experimental evidence before firm conclusions can be made. Experimental studies [34] have shown the kidney to be important in the Pb exchange process between

mussels and their environment. The exchange rate of Pb was also high for the stomach, as was metal uptake by the gills. Uptake of Zn, Mn, Co and Fe followed the order : stomach > gills > foot > mantle > gonad > adductor muscle [36]. Schulz-Baldes [34] proposed a general pattern of trace metal uptake in bivalves, with the kidney, gills and stomach having the highest, and the gonads, mantle and muscle the lowest rate of uptake.

Concentrations of several trace metals in New Zealand mussels were found to be highest in visceral mass and intestine, although the kidney was not isolated [28]. Elevated concentrations of lead were found in the stomach and gills of Port Phillip Bay mussels [27]. Again, no measurements were obtained from the kidney.

Food ingestion has been cited as a greater source of ^{65}Zn, ^{54}Mn, ^{58}Co and ^{59}Fe uptake than sorption from the water for mussels [36]. However, Pb accumulated in the stomach and kidney of mussels at the same rate in Pb-labelled medium experiments as it did for Pb-labelled food experiments [34]. Metals were taken up primarily across gill and adductor muscle membranes, passed into the blood stream and then transported to the kidney and other organs. Similar processes of ion adsorption at water-membrane interfaces and absorption across semi-permeable membranes were postulated by Romeril [22] as important in the experimental accumulation of Zn by oysters. Some doubt exists therefore regarding the pathway of trace metal accumulation in mussels. Measurements of the concentrations of metals in different organs of mussels in their natural habitat are required to enable the determination of metal uptake pathways.

This report investigates the use of the mussel *Mytilus edulis* as an indicator of trace metal concentration in dockyard waters, and presents preliminary results for a range of metals in whole mussels and various organs from mussels at WND.

2. METHOD

2.1 Sample Collection

Mussels were collected from the intertidal zone of pilings at the end of Dockyard Pier, at Williamstown Naval Dockyard, Hobson's Bay. The dockyard waters are estuarine with a mean salinity of 28‰. The water temperature varies seasonally from 10°C to 22°C [32].

Twenty mussels of between 3.5 and 5.0 cm in length were collected and placed in a bucket of ambient seawater for forty-eight hours to allow defecation of gut contents. They were then frozen pending analysis.

2.2 Sample Analysis

The lengths of all mussels were measured. The foot, mantle, gills, posterior adductor muscle, kidney, stomach (including digestive gland) and remaining soft parts were dissected from ten mussels. These organs and the whole soft parts of the other ten mussels were isolated, weighed and freeze-dried to constant weight.

Several methods of digestion [38,39] were tested and the results compared to the biological reference material NBS-SRM 1571 - Orchard Leaves (Table 1). The nitric acid/hydrogen peroxide mixture yielded the most accurate results. The method of Topping [38] was unsuitable as it led to the formation of unstable perchlorate salts.

TABLE 1

COMPARATIVE DIGESTION PROCEDURES FOR BIOLOGICAL TISSUE

Digestion Procedure	Metal Concentration (μg/g) dry wt			
	Cd	Pb	Cu	Zn
NBS Orchard Leaves	0.11 ± 0.02	45 ± 3	12 ± 1	25 ± 3
Nitric Acid	0.79	44.1	15.3	36.3
Nitric/Perchloric Acid	1.11	38.8	14.6	33.1
Nitric Hydrogen Peroxide	0.81	44.1	12.6	31.9

The nitric acid/hydrogen peroxide method subsequently adopted was as follows :

(i) tissue samples were refluxed with 10 ml concentrated nitric acid and evaporated to near dryness,

(ii) samples and a mixture of 10 ml water, 10 ml nitric acid and 10 ml hydrogen peroxide were refluxed and the step repeated until a colourless solution indicated oxidation was complete,

(iii) samples were then evaporated to near dryness and the residue diluted to 25 ml with distilled water.

Fe and Zn concentrations were determined by flame mode analysis in a Varian Techtron Model AA-6 Atomic Absorption Spectrophotometer (AAS). The manufacturers recommendations were used for all instrument settings [40]. Cu, Cd, Pb, Ni, Co, Mn and Ag were analysed by flameless AAS using the Varian Techtron Model 63 carbon rod furnace. Samples were injected into the carbon furnace using a 5 μl teflon-tipped microsyringe. The furnace settings used for all metals are described in Table 2.

TABLE 2

FURNACE SETTINGS FOR ALL TRACE METAL ANALYSES

	Temp (°C)	Time (sec)
Dry	150	30
Ash	400	30
Atomise	2000	4

Analyses were calibrated with standard solutions in the working range of the analytes. Standard addition methods of calibration were not used as the matrix was destroyed prior to analysis of the nitric acid/hydrogen peroxide mixture.

2.3 Data Analysis

The kidney sample from one mussel was lost during preparation. Calculations which involved the kidney were therefore made from only nine samples.

Final trace metal concentrations were calculated in parts per million (ppm) dry weight. The relationship between wet weight (w) and dry weight (D), calculated by linear regression, was $w = 9.01D-0.03$ (correlation coefficient = 0.97).

As the statistical distribution of trace metal concentrations was not known, the Friedman's non-parametric two-way analysis of variance by ranks was used to determine the significance of differences between several groups of data. Where differences occurred, a critical range method was used to test the significance of differences between all possible pairs in the analysis [41].

3. RESULTS AND DISCUSSION

3.1 Trace Metal Uptake by Different Organs

Trace metal concentrations varied significantly ($P < 0.01$) between organs (Table 3). For each metal, pairs of organs which differed significantly ($P < 0.05$) are shown in Table 4. The kidney was the primary site for metal concentration. 25% of Pb, 16% of Co, 15% of Zn and 13% of Ni found in the whole soft tissue were present in the kidney whilst the dry weight of the organ constituted only 2.7% of the total body weight. Similarly, Schulz-Baldes [34] found 50% to 70% of total Pb in the kidney whilst the organ formed only 6% to 8% of the dry weight of mussels sampled in that study.

Some metals, particularly Fe, Cu, Co, Pb and Ni are also strongly concentrated in the stomach.

The results suggest that the various metals may have different accumulation pathways. Higher concentrations of Co, Fe and Cu occurred in the stomach than in the gills and muscle, and these metals may have been taken up primarily during food ingestion. The other metals do not show higher concentrations in the stomach and may be primarily accumulated by sorption processes directly from the water. The high concentrations of metals in the kidney, for all metals, support observations [34] that the metals are transferred to the circulatory systems from either the stomach or gills and transported to the kidney where they are removed from the blood.

Further experimental and *in situ* evidence is required before the uptake pathways of each metal can be determined.

3.2 Comparison with other Areas

Trace metal concentrations from the present investigation are similar in magnitude to those from other studies (Table 5). Whenever possible results from Port Phillip Bay and Westernport Bay, Victoria, have been cited. However, the absence of data for some metals necessitated the inclusion of overseas results. The only data available on Co accumulation by mussels was for *Mytilus galloprovincialis* from a study in the Gulf of Spieza, Italy [30].

Uptake of trace metals by mussels can vary considerably with salinity and temperature of the water [11,43], size and reproductive condition of the mussels [42,44] and season and depth of sampling [11]. Several of these parameters would vary between studies and interstudy comparisons should be made with some reservations.

The concentrations of Zn, Cd, Cu and Pb fall below permissible levels recommended for shell fish by the National Health and Medical Research Council [45]. However, as our results are from a single sampling experiment, an assessment of trace element pollution at WND cannot be made at this time.

3.3 Mytilus edulis as an Indicator Organism in Dockyard Waters

Mussels have been used to assess trace metal levels in both Australia and overseas regions. This investigation has noted the availability of mussels in WND and exemplified the ability of *Mytilus edulis* to take up and concentrate trace metals far above environmental levels. As mussels can also be obtained from Sydney Harbour and Cockburn Sound, they provide an effective monitor of trace metal levels in waters adjacent to the major Australian Naval Facilities.

Where *Mytilus* is to be used as an indicator of trace metal pollution, sampling methods need to be planned to maximise the usefulness of results. Mussel length and depth of collection should be constant, and water temperature and salinity, sampling season and reproductive condition of the mussels recorded. This data would enable valid comparisons of trace metal levels from different studies to be made.

Comparisons of concentrations in organs that respond rapidly to environmental conditions, such as the kidney, to those that react more slowly, such as the foot, can provide additional information on past environmental levels of trace metal concentrations. In addition to the determination of

metals concentrations in whole mussels, future sampling programmes should include measurement of heavy metal concentrations in several organs.

4. CONCLUSIONS

1. A nitric acid/hydrogen peroxide mixture was a suitable digestion agent for heavy metal analyses of mussel tissue.

2. Trace metal concentrations varied significantly between organs of mussels collected from Williamstown Naval Dockyard.

3. Concentrations of Pb, Zn, Cu and Cd in mussels from WND were below those recommended for molluscs by the National Health and Medical Research Council.

4. *Mytilus edulis* satisfies criteria for indicator organisms of trace metals in Australian Naval Dockyard waters.

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TABLE 3

TRACE METAL CONCENTRATION (mean ± s.d., ppm dry weight)
IN *MYTILUS EDULIS*

	Whole Soft Tissue	Gills	Mantle	Foot	Kidney	Stomach	Posterior Adductor Muscle	Soft Remains
Fe	253 ± 99	247 ± 105	499 ± 400	469 ± 388	1453 ± 529	950 ± 278	208 ± 88	290 ± 147
Zn	279 ± 102	560 ± 115	470 ± 981	193 ± 75	1477 ± 519	187 ± 103	145 ± 64	195 ± 56
Cu	10.3 ± 3.1	15.6 ± 5.6	37.5 ± 68.2	27.6 ± 14.6	135 ± 149	41.8 ± 15.7	8.4 ± 7.1	21.3 ± 7.6
Pb	11.4 ± 3.2	31.0 ± 14.6	67.8 ± 52.4	77.3 ± 28.1	506 ± 306	95.0 ± 3.8	27.4 ± 15.1	7.1 ± 3.0
Cd	6.4 ± 2.4	17.2 ± 5.3	22.3 ± 21.9	28.5 ± 14.4	40.9 ± 21.2	12.3 ± 13.1	23.1 ± 31.9	1.1 ± 0.4
Ni	14.3 ± 3.9	74.0 ± 70.6	171.4 ± 196.3	134.6 ± 79	445 ± 259	97.3 ± 45.1	97.4 ± 63.9	13.6 ± 4.3
Mn	7.1 ± 2.0	6.3 ± 3.4	23.3 ± 23.6	9.5 ± 2.2	50.5 ± 17.4	26.8 ± 21.8	23.4 ± 16.5	22.9 ± 6.5
Ag	0.66 ± 0.30	1.14 ± 0.56	3.95 ± 3.02	7.77 ± 8.34	10.6 ± 5.21	2.11 ± 1.53	2.23 ± 1.59	3.5 ± 1.8
Co	4.16 ± 2.48	19.5 ± 6.6	51.4 ± 38.6	42.2 ± 17.6	231 ± 118	72.7 ± 65.2	14.4 ± 7.1	14.7 ± 3.9

TABLE 4

ORGAN PAIRS WITH SIGNIFICANTLY DIFFERENT CONCENTRATIONS OF THE TRACE METALS LISTED

Higher Concentration / Lower Concentration	Kidney	Stomach	Foot	Mantle	Muscle	Gills	Soft Remains
Soft Remains	Fe, Ni, Cd Pb, Cu, Co	Ni, Pb, Co	Ni, Cd, Pb	Ni, Cd	Pb, Cd	Cd	
Gills	Fe, Zn, Ni Pb, Mn, Ag Co	Fe	Ag				Mn
Muscle	Fe, Zn, Ni Pb, Cu, Ag Co	Fe, Cu, Co					
Stomach	Zn, Cd, Ag						
Mantle	Zn, Cu						
Foot	Zn, Mn						

TABLE 5

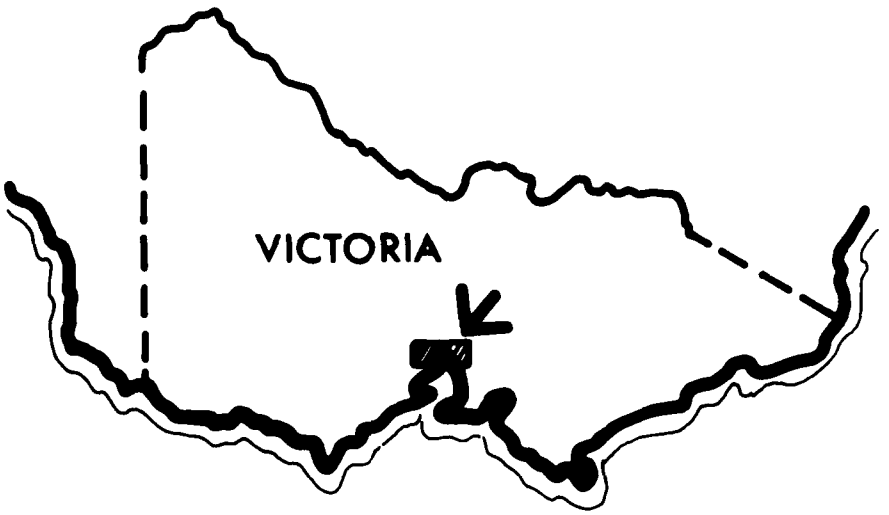
COMPARISON OF TRACE METAL LEVELS (ppm dry wt) IN MUSSELS FROM OTHER STUDIES TO THOSE OBTAINED FROM THIS INVESTIGATION

Metal	Permissible Limit + :	Hobson's Bay		Frankston (PPB) (26)+	Crib Point WPB (10)	Gulf of Spieze Italy (30)*	Tasman Bay, NZ (28)
		Our study	(26)+				
Fe		253			250-465		1960
Zn	9000	279	719	264	212-238	203-379	91
Cu	270	10.3	5.8	3.5	5.6-6.4	6.9-33.7	9
Pb	18	11.4	16.6	5.8	0.5	13.9-44.6	12
Cd	18	6.4	13.4	3.8	0.6-0.9	2.0-6.8	10
Ni		14.3				1.3-10.9	7
Mn		7.1			5.3-6.2	11.8-37.8	27
Ag		0.7					0.1
Co		4.2				0.8-3.2	

\+ Calculated from wet weight

: Recommended by National Health and Medical Research Council

* *Mytilus galloprovincialis*



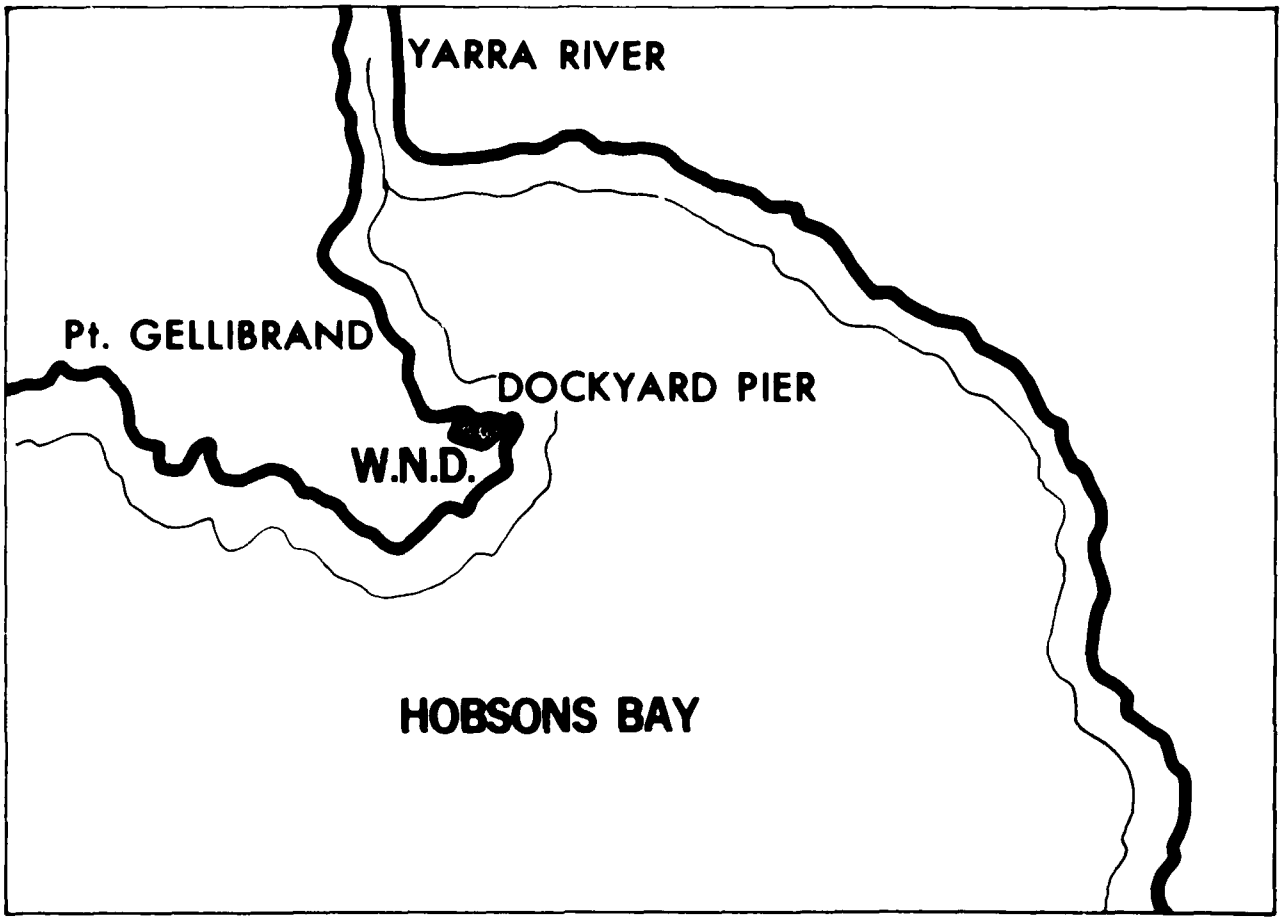


FIG. 1 - Locality Diagram

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